

IN THE CLAIMS:

1.-83. (cancelled)

84. (new) A method for amplifying a target nucleic acid sequence comprising a target nucleic acid, the method comprising the steps of:

- a) hybridizing a riboprimers to a DNA template comprising the target nucleic acid sequence;
- b) extending the riboprimers with a DNA polymerase; and
- c) cleaving the annealed riboprimers with an RNase H enzyme such that another riboprimers hybridizes to the template and repeats primer extension, whereby multiple copies of the complementary sequence of the target nucleic acid sequence are produced.

85. (new) The method of claim 84, further comprising, prior to step (b), the step of hybridizing a blocking oligonucleotide to a region of the template that is 5' with respect to hybridization of the riboprimers to the template.

86. (new) The method of claim 84, wherein the method is conducted under isothermal conditions.

87. (new) The method of claim 84, further comprising the step of:

- d) attaching the multiple copies produced in step c) onto a solid substrate to make a microarray of the multiple copies.

88. (new) The method of claim 84, further comprising the step of hybridizing the multiple copies produced in step c) to a microarray of nucleic acid molecules immobilized on a surface of a solid phase.

89. (new) The method of claim 84, wherein step (b) comprises utilization of at least one type of labeled dNTP such that labeled extension products are generated.

90. (new) The method of claim 84, wherein the riboprimers comprises AMP, GMP, 2'-F-dUMP, and 2'-F-dCMP.

91. (new) The method of claim 84, wherein a plurality of riboprimers is used.

92. (new) The method of claim 84, wherein the riboprimers comprises only ribonucleotides.

93. (new) The method of claim 84, wherein the riboprimers comprises at least one pyrimidine 2'-deoxyribonucleotide having a 2'-substituent on the sugar moiety, which 2'-substituent is selected from the group consisting of a fluorine substituent, an amino substituent, a methoxy substituent, and an azido substituent.

94. (new) The method of claim 84, wherein the riboprimers comprises purine ribonucleotides and pyrimidine 2'-fluoro-2'-deoxyribonucleotides.

95. (new) The method of claim 84, wherein the entire sequence of the riboprimers is complementary to the 3'-end portion of the target sequence.

96. (new) The method of claim 84, wherein the 5'-end portion of the riboprimers is not complementary to the target nucleic acid sequence.

97. (new) The method of claim 96, wherein the non-complementary 5' end portion provides a sequence that can be copied by second-strand primer extension using as a template a first-strand cDNA primer extension product made using the riboprimers.

98. (new) The method of claim 96 wherein the target-complementary sequence at the 3'-end portion of the riboprimers comprises a randomized sequence.

99. (new) The method of claim 85, wherein the blocking oligo comprises a peptide nucleic acid (PNA).

100. (new) The method of claim 84, wherein the DNA polymerase is selected from the group consisting of BST DNA polymerase large fragment (ISOTHERM), SEQUITHERM DNA polymerase (EPICENTRE Technologies), BCABEST DNA polymerase (Takara Shuzo Co.), phi29 DNA polymerase, and Exo-minus Klenow DNA polymerase.

101. (new) The method of claim 84, wherein the RNase H enzyme is a thermostable RNase H.

102. (new) The method of claim 101, wherein said thermostable RNase H is selected from the group consisting of HYBRIDASE thermostable RNase H (EPICENTRE Technologies), Tth RNase H, and Tfl RNase H.

103. (new) The method of claim 84, wherein said RNase H is *E. coli* RNase H.

104. (new) A method for amplifying a target nucleic acid sequence comprising a target nucleic acid, the method comprising:

- a) obtaining a DNA comprising a target nucleic acid sequence;
- b) obtaining a riboprimers, the riboprimers comprising ribonucleotides, wherein at least the 3'-end portion of the riboprimers is complementary to a portion of the target nucleic acid sequence;
- c) annealing the riboprimers to the single-stranded DNA;
- d) obtaining a strand-displacing DNA polymerase;
- e) primer extending the riboprimers annealed to the DNA with the strand-displacing DNA polymerase under strand-displacing polymerization conditions;
- f) obtaining a double-stranded complex comprising the DNA and a primer extension product, wherein the primer extension product comprises the riboprimers sequence in its 5'-end portion and the target sequence in its 3'-end portion;

- g) contacting the double-stranded complex with an RNase H enzyme under enzyme reaction conditions so as to release at least a portion of the riboprimers sequence in the 5'-end portion of the primer extension product of the double-stranded complex;
- h) annealing a second riboprimers to the single-stranded DNA of the double-stranded complex, wherein the second riboprimers anneals to the single-stranded DNA at the position where the portion of the riboprimers sequence of the primer extension product was released;
- i) primer extending the second riboprimers annealed to the single-stranded DNA of the double-stranded complex with the strand-displacing DNA polymerase under strand-displacing polymerization conditions, so as to displace the first primer extension product from the double-stranded complex and obtain a second double-stranded complex comprising the single-stranded DNA and a second primer extension product; and
- j) obtaining the primer extension product that was displaced from the double-stranded complex as a result of extending the second riboprimers annealed to the single-stranded DNA.

105. (new) The method of claim 104, further comprising the step of annealing a blocking oligo to a region of the DNA, wherein the 5'-end of the blocking oligo that is annealed to the single-stranded DNA delimits the 3'-end of the target nucleic acid sequence.

106. (new) The method of claim 104, further comprising step l) repeating steps b through j whereby multiple copies of the primer extension products corresponding to the target sequence are produced.

107. (new) The method of claim 104 further comprising step l) detecting the primer extension products produced.

108. (new) The method of claim 104, further comprising step l) quantifying the primer extension products.

109. (new) The method of claim 104, wherein a plurality of riboprimers is used.

110. (new) The method of claim 104, wherein the riboprimers comprises only ribonucleotides.

111. (new) The method of claim 104, wherein the riboprimers comprises at least one pyrimidine 2'-deoxyribonucleotide having a 2'-substituent on the sugar moiety, which 2'-substituent is selected from the group consisting of a fluorine substituent, an amino substituent, a methoxy substituent, and an azido substituent.

112. (new) The method of claim 104, wherein the riboprimers comprises purine ribonucleotides and pyrimidine 2'-fluoro-2'-deoxyribonucleotides.

113. (new) The method of claim 104, wherein the entire sequence of the riboprimers is complementary to the 3'-end portion of the target sequence.

114. (new) The method of claim 104, wherein the 5'-end portion of the riboprimers is not complementary to the target nucleic acid sequence.

115. (new) The method of claim 114, wherein the non-complementary 5' end portion provides a sequence that can be copied by second-strand primer extension using as a template a first-strand cDNA primer extension product made using the riboprimers.

116. (new) The method of claim 114, wherein the target-complementary sequence at the 3'-end portion of the riboprimers comprises a randomized sequence.

117. (new) The method of claim 105, wherein the blocking oligo comprises a peptide nucleic acid (PNA).

118. (new) The method of claim 104, wherein the DNA polymerase is selected from the group consisting of BST DNA polymerase large fragment (ISOTHERM), SEQUITHERM DNA polymerase (EPICENTRE Technologies), BCABEST DNA polymerase (Takara Shuzo Co.), phi29 DNA polymerase, and Exo-minus Klenow DNA polymerase.

119. (new) The method of claim 104, wherein the RNase H enzyme is a thermostable RNase H.

120. (new) The method of claim 119, wherein said thermostable RNase H is selected from the group consisting of HYBRIDASE thermostable RNase H (EPICENTRE Technologies), Tth RNase H, and Tfl RNase H.

121. (new) The method of claim 104, wherein said RNase H is *E. coli* RNase H.

122. (new) A method of generating multiple copies of a polynucleotide sequence complementary to an RNA sequence of interest, the method comprising the steps of:

- a) extending a first primer hybridized to a target RNA with an RNA-dependent DNA polymerase, wherein the first primer is a riboprimers, whereby a complex comprising a first primer extension product and the target RNA is produced;
- b) cleaving RNA in the complex of step (a) with an RNase H enzyme;
- c) extending a second primer hybridized to the first primer extension product with a DNA-dependent DNA polymerase, whereby a second primer extension product is produced to form a complex of first and second primer extension products;
- d) cleaving the riboprimers in the complex of first and second primer extension products with an RNase H enzyme such that a riboprimers hybridizes to the second primer extension product; and
- e) extending the riboprimers hybridized to the second primer extension product with a DNA-dependent DNA polymerase,

whereby the first primer extension product is displaced and multiple copies of a polynucleotide sequence complementary to the RNA sequence of interest are generated.

123. (new) The method of claim 122, wherein the RNA-dependent DNA polymerase is selected from the group consisting of Bst DNA polymerase, BST DNA polymerase large fragment (ISOTHERM), Moloney murine leukemia virus (MMLV) reverse transcriptase, and avian myeloblastosis virus (AMV) reverse transcriptase.

124. (new) The method of claim 123, wherein said MMLV reverse transcriptase comprises RNase H minus MMLV reverse transcriptase.

125. (new) The method of claim 122, wherein a plurality of riboprimers is used to generate multiple copies of a polynucleotide sequence complementary to the RNA sequence of interest.

126. (new) The method of claim 122, wherein a plurality of different riboprimers is used for hybridizing to the target RNA.

127. (new) The method of claim 122, wherein the riboprimer comprises only ribonucleotides.

128. (new) The method of claim 122, wherein the riboprimer comprises at least one pyrimidine 2'-deoxyribonucleotide having a 2'-substituent on the sugar moiety, which 2'-substituent is selected from the group consisting of a fluorine substituent, an amino substituent, a methoxy substituent, and an azido substituent.

129. (new) The method of claim 122, wherein the riboprimer comprises purine ribonucleotides and pyrimidine 2'-fluoro-2'-deoxyribonucleotides.

130. (new) The method of claim 122, wherein the riboprimer comprises AMP, GMP, 2'-F-dUMP, and 2'-F-dCMP.

131. (new) The method of claim 122, wherein the riboprimers that hybridizes to the target RNA comprises a poly-U sequence.

132. (new) The method of claim 122, wherein the riboprimers that hybridizes to the target RNA is a random primer.

133. (new) The method of claim 122, wherein the riboprimers that hybridizes to the target RNA comprises a 5'-portion that is not hybridizable to the target RNA under conditions under which the riboprimers hybridizes to the target RNA.

134. (new) The method of claim 122, wherein the target RNA is mRNA.

135. (new) The method of claim 122, wherein the RNase H enzyme is a thermostable RNase H.

136. (new) The method of claim 135, wherein said thermostable RNase H is selected from the group consisting of HYBRIDASE thermostable RNase H (EPICENTRE Technologies), Tth RNase H, and Tfl RNase H.

137. (new) The method of claim 122, wherein said RNase H is *E. coli* RNase H.

138. (new) The method of claim 122, wherein the DNA polymerase is selected from the group consisting of BST DNA polymerase large fragment (ISOTHERM), SEQUITHERM DNA polymerase (EPICENTRE Technologies), BCABEST DNA polymerase (Takara Shuzo Co.), phi29 DNA polymerase, and Exo-minus Klenow DNA polymerase.

139. (new) The method of claim 122, wherein the second primer is a random primer.

140. (new) The method of claim 122, wherein the second primer comprises a fragment of the target RNA hybridized to the primer extension product, which fragment is

generated by cleaving RNA in the complex in step (b) with an enzyme that cleaves RNA from an RNA/DNA hybrid.

141. (new) The method of claim 122, wherein the second primer comprises DNA.

142. (new) The method of claim 122, wherein the RNA-dependent DNA polymerase and DNA-dependent polymerase are the same enzyme.

143. (new) The method of claim 140 wherein the RNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA hybrid are the same enzyme.

144. (new) The method of claim 122, further comprising the step of:

f) generating multiple copies of a polynucleotide sequence complementary to two or more different sequences of interest.

145. (new) The method of claim 144, wherein at least two different riboprimers that hybridize to the target RNA are used

146. (new) The method of claim 122, further comprising the step of:

f) attaching the multiple copies onto a solid substrate to make a microarray of the multiple copies.

147. (new) The method of claim 122, further comprising the step of:

e) hybridizing the multiple copies to a microarray of nucleic acid molecules immobilized on a surface of a solid phase.